European Journal of Medicinal Chemistry 82 (2014) 120–126

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Preliminary communication

Group A streptococcus inhibitors by high-throughput virtual screening

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ARTICLE INFO

Article history:
Received 15 December 2013
Received in revised form
29 April 2014
Accepted 1 May 2014
Available online 4 May 2014

Keywords:
Group A streptococcus
RNase J1/J2
Homology model
High-throughput virtual screening (HTVS)

ABSTRACT

Group A streptococcus (GAS) is a Gram-positive bacterium, which can cause multiple types of disease from mild infections of skin and throat to invasive and life-threatening infections. Recently RNase J1 and J2 were found to be essential for the growth of GAS. In order to identify inhibitors against RNase J1/J2, homology models of both the ligand-free apo-form and the ligand-bound holo-form complexes were constructed as templates for high-throughput virtual screening (HTVS). A focused small molecule library and the commercially available Maybridge database were employed as sources for potential inhibitors. A cell-based biological assay identified two compounds with 10 μM MIC activity.

1. Introduction

Streptococcus pyogenes, also known as group A streptococcus (GAS), is a Gram-positive bacterium and one of the most frequent pathogens in humans. It is estimated that about 5–15% of normal individuals harbor the bacterium, usually in the respiratory tract without signs of disease [1]. Most GAS infections are relatively mild illnesses such as streptococcal sore throat (strep throat) or streptococcal skin infections (impetigo). However, GAS can also cause more serious conditions such as scarlet fever, rheumatic fever, postpartum fever, wound infections and pneumonia [2]. Occasionally these bacteria can cause severe and even life-threatening diseases. Termed "invasive GAS disease", the latter occur when bacteria invade parts of the body where they are usually not found, such as the blood, muscle or lungs [3]. Two of the most severe forms of invasive GAS are necrotizing fasciitis and streptococcal toxic shock syndrome. Necrotizing fasciitis, occasionally described by the media as "the flesh-eating bacteria", is a rapidly progressive disease which destroys muscles, fat and skin tissue. On the other hand, streptococcal toxic shock syndrome (STSS) results in a rapid drop in blood pressure as organs such as the kidney, liver and lungs begin to fail. While 10%–15% of untreated patients with invasive GAS disease die from their infection, approximately 25% of patients with necrotizing fasciitis and more than 35% with STSS experience the same fate [3]. Current treatments for GAS include oral or IV injection of antibiotics such as penicillin and surgical removal of dead tissues. Early treatment can reduce the risk of death from invasive disease, but it cannot prevent death in every case. In addition, an increasing number of GAS strains are being reported to show resistance to one or more antibiotics. Although there are no GAS strains resistant to penicillin at present, treatment with this antibiotic fails to eradicate GAS infections in up to a third of all cases [4]. Therefore, the development of new drugs is crucial.

The ability of GAS to produce different diseases and to infect the host results from its ability to regulate expression of virulence factors required for attachment to host tissues, evade the host immune response and spread rapidly throughout the host [5–7]. Because of its importance in disease progression, regulation of gene expression in GAS has been studied, and it is believed that control of mRNA decay is an important mechanism of this regulation. Recently, Bugrysheva and Scott found that RNase J1 and J2 are essential for growth of GAS and play independent roles in mRNA decay of the organism [8]. Therefore, if small molecules can be

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http://dx.doi.org/10.1016/j.ejmech.2014.05.006
0223-5234/

identified to inhibit RNase J1/J2, GAS growth should be terminated. To date, while several strategies for mediating GAS have been described [9–12], attempts to block RNase J1 or J2 have not appeared in the literature.

Since neither crystal structures of RNase J1/J2 in GAS nor known inhibitors of the enzymes had been reported, during the course of our work, we employed two computational tools in an effort to overcome the deficits. The first is homology modeling, in which an atomic-resolution model of the "target" protein is constructed from its amino acid sequence and an experimental three-dimensional structure of a related homologous protein. In this study, we prepared a homology model of GAS RNase J1 based on the X-ray crystal structure of *Thermus thermophilus* RNase J. The second tool is high-throughput virtual screening, in which a large library of drug-like chemicals is screened computationally against the receptor (i.e. homology model) followed by experimental assay of those compounds predicted to bind well. Although the physical screening of chemicals against a biological target is one dominant technique in drug discovery, high-throughput virtual screening has also been shown to be successful in predicting new ligands along with their receptor-bound structures, sometimes with high hit rates [13–15]. Herein, we demonstrate a high-throughput virtual screening approach devoted to identification of RNase J1 inhibitors in GAS.

2. Results and discussion

2.1. RNase J target construction

2.1.1. Analysis of the crystal structure of T. thermophilus RNase J

The crystal structure of *T. thermophilus* RNase J has been reported by de la Sierra-Gallay et al. [16] both as the free enzyme (2.3 Å resolution, PDB (Protein Data Bank) code 3BK1) and as a complex with uridine monophosphate (UMP, 2.1 Å, PDB code 3BK2; Fig. S1a, Supporting Information). Structural analysis indicates that *T. thermophilus* RNase J is composed of three distinct domains: a β-lactamase domain which presents in all metallo-β-lactamases, a β-CASP domain which is found only in the β-CASP subfamily of β-lactamases, and a C-terminal domain which is present only in RNase J orthologues (Fig. S1b, Supporting Information). The enzyme's catalytic center, where RNA is hydrolyzed, is located in the cleft between its β-lactamase and β-CASP domains. The catalytic center is formed by several conserved histidines and aspartic acids that bind two Zn^{2+} cations as in most β-lactamases. In *T. thermophilus* RNase J, Asp172 bridges both Zn^{2+} cations as does a Zn-coordinating water molecule. The latter simultaneously establishes a hydrogen bond with His376. His75, His77 and His150 provide three ligands to Zn-1, while His80, His398 and Asp79 ligate Zn-2. Asp204 maintains the orientation of His376 through a hydrogen bond in a manner similar to the hydrogen bond formed between Asp38 and His80. Within the catalytic center of the free enzyme, a sulfate ion resides in a site identical to that of the phosphate of bound UMP forming either water-bridged or direct hydrogen bond interactions with His372, Ser340, Ser374 and Ser151 (Fig. S1c and S1d in the Supporting Information). For the purpose of coupling the homology models with the accompanying screening strategy (see below), we define this sulfate site together with the catalytic center as the "catalytic center binding pocket".

2.1.2. Sequence alignments

The sequences of GAS RNase J1 and J2 obtained from the NCBI (protein accession numbers: AAM80227.1 and AAM79264.1) contain 560 and 553 amino acids, respectively. They were aligned with the sequence of *T. thermophilus* RNase J using the EBI MUSCLE multi sequence alignment protocol [17]. The alignment is shown in Fig. S2 in the Supporting Information. In this alignment, RNase J1 and J2 of GAS share 39%, 33% identities and 59%, 50% similarities with *T. thermophilus* RNase J. Investigating the alignment further, we found the residues which comprise the catalytic center including histidines 75, 77, 80, 150, 372, 376, 398, aspartates 38, 79, 204, 172 and serines 151, 340, 374 are highly conserved in both RNase J, J1 and J2. The residues are organized into sequence motifs I–VIII. Considering that the proposed ligand binding pocket is deeply buried within the cleft between β-CASP and β-lactamase domains, and all the conserved motifs reside in the β-CASP and β-lactamase domains, the C-terminal domain of RNase J was removed and the remaining sequences were realigned with MUSCLE. With the new sequence alignment, the identities between RNase J1 and RNase J and between RNase J2 and RNase J increased to 44% and 38%, respectively.

2.1.3. Homology models of RNase J1

Homology modeling was performed with MODELLER version 9.1 [18] using both the apo-form (two Zn^{2+} cations and a sulfate in the proposed binding pocket) and the UMP complex holo-form (UMP and two Zn^{2+} cations) of *T. thermophilus* RNase J as templates. Model construction included complete backbone and side chain building, loop building and verification of model quality. The crystallized ligands including Zn^{2+} cations, sulfate and UMP were preserved during homology model construction. However, all water molecules inherited from the crystal structure were removed in order to permit potential inhibitory ligands to coordinate with Zn^{2+} at the catalytic site. Were one to retain these water molecules, including the ones coordinated to the Zinc cation, ligand docking in the vicinity of the metal atoms would be blocked. The stereochemical and energetic parameters of the initial 3D protein models were evaluated by PROCHECK [19] and Protein Report in Maestro [20]. Prime version 2.2 [21] embedded in the Schrodinger Suite was used to optimize loop conformations, side chain rearrangement and the placement of insertions and deletions while respecting the template structure context and conservation of structural features with a functional role. The final model was analyzed a second time with PROCHECK for violations of main chain Phi/Psi dihedral bond angle ratios and backbone/side chain steric conflicts (Table S2, Supporting Information). To verify the accuracy of the binding site of the homology model, the water molecules in the binding pocket of the *T. thermophilus* RNase J crystal structure were merged into the GAS RNase J1 homology model and optimized to avoid conflict with the protein by using Protein Preparation Wizard in the Schrodinger Suite. UMP was docked back into the homology model with Glide 5.6 [22] and superposed with the 3BK2 crystal structure to show that the ligand-bound model binding site and the UMP ligand match with a heavy-atom RMSD = 0.38 Å. It should be noted that for the virtual screening discussed below, the water molecule was removed in the 3D docking step in order to allow potential ligands to coordinate with the zinc cation. Figures were generated using Pymol 0.99rc6 [23].

2.1.4. Structure comparisons

Two homology models were generated for RNase J1: the apo-form with two Zn^{2+} cations and a sulfate in the proposed binding pocket and the holo-form complex including UMP and two Zn^{2+} cations. For RNase J1, the root mean square (RMS) deviation between the backbone atoms of the two models and their corresponding templates are both less than 0.4 Å (Fig. 1a and b). In both models, conformations of the His and Asp side chains around the Zn^{2+} cations are highly conserved. Due to the absence of water molecules in the homology model, each Zn^{2+} is surrounded by 4 ligands. An exception is the second zinc cation in the holo-form model that exhibits tri-coordination resulting from side chain flipping of Asp80 [24] (Fig. 2a).